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<p>(21) International Application Number: PCT/GB91/01969</p> <p>(22) International Filing Date: 8 November 1991 (08.11.91)</p> <p>(30) Priority data: 9024282.7 8 November 1990 (08.11.90) GB 9115410.4 17 July 1991 (17.07.91) GB</p> <p>(71) Applicant (for all designated States except US): UNIVERSITY COLLEGE LONDON [GB/GB]: 5 Gower Street, London WC1E 6HA (GB).</p> <p>(72) Inventors: and (75) Inventors/Applicants (for US only): ROOK, Graham, Arthur, William [GB/GB]: Old Hall, Old Hall Road, Steeple Bumpstead, Javer Hill, Suffolk CB9 7EJ (GB). STANFORD, John, Lawson [GB/GB]: Millhouse, Claygate, Marden, Kent TN12 9TE (GB).</p>	<p>(74) Agents: COLLIER, Jeremy, Austin, Grey et al.: J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).</p> <p>(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN (OAPI patent), SU*, TD (OAPI patent), TG (OAPI patent), US.</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	

(54) Title: **MYCOBACTERIUM AS ADJUVANT FOR ANTIGENS**

(57) Abstract

Immunoregulatory material from a mycobacterium other than *M. tuberculosis*, especially killed cells of *M. vaccae*, is an advantageous adjuvant for administration with antigens (including allergens).

40

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

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Mycobacterium as adjuvant for antigens

The present invention relates to carriers, and more particularly adjuvants, for antigens (including allergens), for use in vaccination, and other ways of altering, in a favorable way, the immune response to an antigen.

Killed cells of M. vaccae are known to be useful as immunotherapeutic agents in mycobacterial diseases such as tuberculosis and leprosy (see GB-A-2156673). This known use of M. vaccae may rely upon the stimulation of T-cell mediated immunity to endogenous antigens of M. vaccae. Killed cells of M. vaccae are also useful in the treatment of various autoimmune diseases including rheumatoid arthritis, ankylosing spondylitis and Reiter's syndrome (see PCT/GB 85/00183).

The present invention is founded upon the surprising observation that killed cells of M. vaccae can be used to stimulate and/or modify in a favorable way the immune response to antigens which are not endogenous to M. vaccae.

The immune response to an antigen has two distinct aspects: (1) selection of an epitope (antigen fragment) as an initiator of, and target for, the response; and (2) selection of a particular immune response mechanism as the response directed against the particular epitope selected. Current methods of stimulating the immune response, e.g.

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vaccination, have generally concentrated on the first aspect, but it has become clear, in the light of recent research, that it is essential that the immune response shall be stimulated or modified to a favorable way, since it is possible to modify the immune response unfavorably, leading for example to increased susceptibility to infection. One of the surprisingly beneficial properties of killed cells of M. vaccae is that they promote selection of a favorable immune response mechanism.

10 It is known that, at least in the mouse (see, e.g. Mosmann & Moore, Immunology Today, 1991, A49-A53), different T-cell subsets have different patterns of cytokine secretion. TH2 cells express interleukin(IL)-4, IL-5 and IL-10, whereas TH1 cells produce IL-2, γ -interferon (IFN- γ) and lymphotoxin. The TH2 cells are involved in the pattern of immune responses seen in, e.g., asthma, pollen allergies, and eczema, while TH1 cells are involved in the pattern used in killing intracellular parasites. It appears that killed cells of M. vaccae promote the immune response characteristic of TH1 cells.

20 Conversion of the T cell component of the response to allergens from the TH2 pattern to the TH1 pattern reduces or terminates symptoms of conditions such as asthma, hay fever, and atopic eczema, by reducing production of IgE, reducing recruitment of eosinophils and

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mast cells to the inflamed site, and greatly increasing the antigen concentration required to trigger a response (because the T_H1 response requires a much higher concentration of antigen to be triggered than the T_H2 response). Consequently the levels of allergen in the environment become insufficient to trigger symptoms.

It also appears that killed cells of M. vaccae may promote the immune response characteristic of T_H1 cells, and in the case of autoantigens, enhance reduction of the response via the immunoregulatory network.

The beneficial effect of using killed M. vaccae as an adjuvant may also be associated with the 65 kDa mycobacterial heat shock protein (hsp 65) described by Young et al. "Stress proteins are immune targets in leprosy and tuberculosis", Proc. Natl. Acad. Sci. U.S.A. 85 (1988), pp4267-4270 in form obtained from M. bovis. The preferred autoclaved M. vaccae cells used in the present invention as described below are believed to provide an effective package of adjuvant, hsp 65 and other substances.

The immunoregulatory material derived from M. vaccae or another mycobacterium other than M. tuberculosis may be administered with or separately from the antigen exogenous to the mycobacterium to achieve an improved response to the antigen.

M. tuberculosis is the causative agent of tuberculosis and an avirulent variant of it is used in the

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production of the BCG used vaccine against tuberculosis in immunization programmes throughout the world.

Immunoregulatory material from M. tuberculosis should not be used in accordance with the present invention in order
5 to avoid compromising the use of BCG vaccine by inducing tuberculin test positivity or reducing the subsequent efficacy of BCG. For these reasons the use of immunoregulatory material from M. tuberculosis is excluded from the present invention.

10 It is believed that material from mycobacterial species other than M. tuberculosis might be useful in accordance with the present invention. However, especially as it is already a known immunotherapeutic agent, immunoregulatory material from M. vaccae is currently
15 preferred.

The invention accordingly provides a product comprising immunoregulatory material derived from a mycobacterium other than M. tuberculosis and an antigen exogenous to the mycobacterium as a combined preparation
20 for simultaneous, separate or sequential use for promoting T cell-mediated response to said antigen.

The product of the invention conveniently, and therefore preferably, comprises dead cells of M. vaccae, most preferably cells which have been killed by autoclaving
25 or by irradiation. The product normally comprises more than 10^8 microorganisms per ml of diluent, and preferably

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from 10^8 to 10^{11} killed M. vaccae microorganisms per ml of diluent.

The diluent may be pyrogen-free saline for injection alone, or a borate buffer of pH 8.0. The diluent
5 should be sterile. A suitable borate buffer is:

Na ₂ B ₄ O ₇ ·10H ₂ O	3.63 g
H ₃ BO ₃	5.25 g
NaCl	6.19 g
Tween 80	0.0005%
10 Distilled Water	to 1 litre

The preferred strain of M. vaccae is one denoted R877R isolated from mud samples from the Lango district of Central Uganda (J.L. Stanford and R.D. Paul, Ann. Soc. Belge Med, Trop. 1973, 53 141-389). The strain is a stable
15 rough variant and belongs to the aurum sub-species. It can be identified as belonging to M. vaccae by biochemical and antigenic criteria (R. Bonicke, S.E. Juhasz., Zentr albl. Bakteriол. Parasitenkd. Infection skr. Hyg. Abt. 1, Orig., 1964, 192, 133).

20 The strain denoted R877R has been deposited under the Budapest Convention at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

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For the preparation of the product of the invention, the microorganism M. vaccae may be grown on a suitable solid medium. A modified Sauton's liquid medium is preferred (S.V. Boyden and E. Sorkin., J. Immunol, 1955 5 75, 15) solidified with agar. Preferably the solid medium contains 1.3% agar. The medium inoculated with the microorganisms is incubated aerobically to enable growth of the microorganisms to take place, generally at 32°C for 10 days. The organisms are harvested, then weighed and 10 suspended in a diluent. The diluent may be unbuffered saline but is preferably borate-buffered and contains a surfactant such as Tween 80 as described above. The suspension is diluted to give 200 mg of microorganism/ml. For further dilution, borate buffered saline is preferably 15 used so that the suspension contains 10 mg wet weight of microorganisms/ml of diluent. The suspension may then be dispensed into suitable multidose vials (e.g. 1 ml). Although the microorganisms in the vials may be killed using irradiation, e.g. from ⁶⁰Cobalt at a dose of 2.5 20 megarads, or by any other means, for example chemically, it is preferred to kill the microorganisms by autoclaving, for example at 10-15 psig (69-104 kPa) for 10-15 minutes (115°- 125°C). It has been discovered, unexpectedly, that autoclaving yields a more effective preparation than 25 irradiation.

Extracts or fractioned portions of the

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microorganisms can also be used provided, of course, they have the required adjuvant effect.

The immunotherapeutic product of the invention comprises an association of an effective, non-toxic immunomodifying amount of an immunoregulatory material from a mycobacterium other than M. tuberculosis, especially M. vaccae, and of an effective, non-toxic, immunity-stimulating amount of an antigen exogenous to the mycobacterium.

10 The exogenous antigen may be any antigen against which it is desired to stimulate T-cell mediated immunity or to alter the nature of the T-cell response, to achieve palliation or cure of the infection or other condition to be treated. Examples include antigens associated with
15 diseases at present regarded as having an autoimmune aetiology such as multiple sclerosis, antigens associated with chronic viral infections such as hepatitis, bovine spongiform encephalopathy (BSE), and myoencephalitis (ME), antigens associated with cryptic parasite infections such
20 as leishmaniasis and trypanosomiasis, and allergens (e.g. those present in pollens, animal dander, and house dust mite) responsible for such conditions as hayfever, asthma, food allergy and eczema. The immunotherapeutic product of the invention incorporating the appropriate exogenous
25 antigen may be used prophylactically or therapeutically.

The exogenous antigen may be produced by any

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conventional technique, such as by culture and killing or attenuating the disease organism to provide a killed or attenuated vaccine, by separation and purification of the antigen, with optional chemical modification thereof, from
5 a disease organism or, in the case of proteinaceous antigens, by expression of a gene encoding the antigenic protein in a suitable recombinant organism.

The exogenous antigen may be combined with the immunoregulatory mycobacterial material by admixture,
10 chemical conjugation or adsorption using conventional techniques. Alternatively the exogenous antigen may be produced by expression of an exogenous gene (for instance contained within a plasmid, cosmid, viral or other expression vector or inserted into the genome of the
15 mycobacteria) in the mycobacteria from which the immunoregulatory material is also produced. Thus, for instance, recombinant M. vaccae may be cultured so as to achieve expression of the exogenous antigen and then killed and processed as described above, or under such conditions
20 appropriately modified to preserve the biological activity of the exogenous antigen, to provide an immunoregulatory material containing the exogenous antigen. Techniques for obtaining and expressing such exogenous genes are conventional.

25 The therapeutic agent is in general administered by injection in a volume in the range 0.1-0.2 ml, preferably

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0.1 ml, given intradermally. A single dosage will in general contain from 10^7 to 10^{10} killed M. vaccae microorganisms. It is preferred to administer to patients a single dose containing 10^8 to 2×10^9 killed M. vaccae.

5 However, the dose may be repeated depending on the condition of the patient.

The amount of exogenous antigen administered in association with the M. vaccae is in general the same amount as has previously been used when the given antigen has been administered to provide an immune response. In the case of antigens involved in hay fever and asthma, the required dosage depends on the manner in which the antigen is extracted and specific dosages which are generally applicable cannot be given, although therapeutic preparations containing such antigens are well known, see the article on "Desensitising vaccines", Brit. Med. J. 293 (1986) p.948. For other types of antigen not involved in hay fever or asthma, the usual dosage is in the range of 0.1 to 5 μ g.

20 The therapeutic agent may be administered with the antigen, typically in admixture, but it is within the scope of the invention to administer, e.g. by injection, first the therapeutic agent, e.g. killed cells of M. vaccae, and then, into the same site, the exogenous antigen.

25 Although the therapeutic agent will generally be administered by intradermal injection, other routes, e.g.

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oral administration, can also be used.

The invention includes within its scope a method of treatment of the human or animal body which comprises administering an effective non-toxic amount of
5 immunoregulatory material derived from a mycobacterium other than M. tuberculosis and, with or following the said material, of an antigen exogenous to the mycobacterium to a human or animal in need of T-cell mediated immunity against the exogenous antigen or otherwise in need of the pattern
10 of T-cell mediated response against the exogenous antigen promoted by the said immunoregulatory material.

The invention further provides the use, in the manufacture of an immunotherapeutic composition for use in treatment of the human or animal body by promoting the T-
15 cell mediated response to an exogenous antigen, of immunoregulatory material derived from a mycobacterium other than M. tuberculosis, and pharmaceutical formulations comprising an association of the said immunoregulatory material and an antigen exogenous to the mycobacterium and
20 one or more diluents or carriers therefor.

The pharmaceutical formulation can contain further ingredients such as additional adjuvants, preservatives, stabilisers etc. It may be supplied in sterile injectable liquid form or in sterile freeze-fried form which is
25 reconstituted prior to use.

The following Example illustrates the invention.

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EXAMPLE

M. vaccae NCTC 11659 is grown on a solid medium comprising modified Sauton's medium solidified with 1.3% agar. The medium is inoculated with the microorganism and
5 incubated for 10 days at 32°C to enable growth of the microorganism to take place. The microorganisms are then harvested by gently scraping the surface of the agar and weighed (without drying) and suspended in M/15 borate buffered saline at pH8 to give 10 mg of microorganisms/ml
10 of saline. The suspension is dispensed into 5 ml vials, and then autoclaved for 15 minutes at 15 psi (104 kPa) and about 120°C to kill the microorganisms. This is then dispensed into suitable multidose vials. After cooling, 1/10th volume of exogenous antigen (at the standard
15 concentration of 2µg/ml) is added. The therapeutic agent thus produced is stored at 4°C before use. A single dose consists of 0.1 ml of the suspension, which should be shaken vigorously immediately before use, containing 1 mg wet weight of M. vaccae and 0.02 µg of exogenous antigen.
20 The dose is given by intradermal injection normally over the left deltoid muscle.

Only one dose is normally required. The patient should not receive high dose steroids or other immuno-suppressive therapy. Up to six months may elapse before the beneficial
25 effect becomes apparent.

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CLAIMS

1. A product comprising immunoregulatory material derived from a mycobacterium other than M. tuberculosis and an antigen exogenous to the mycobacterium as a combined preparation for simultaneous, separate or sequential use for promoting T cell-mediated response to said antigen.
2. A product according to claim 1, wherein the immunoregulatory material derived from a mycobacterium comprises dead cells of M. vaccae.
3. A product according to claim 2, wherein the cells of M. vaccae have been killed by autoclaving.
4. A product according to claim 2 or claim 3, wherein the immunoregulatory material derived from M. vaccae comprises the 65 kDa heat shock protein.
5. A product according to any one of claims 2 to 4 wherein the material derived from M. vaccae is derived from the strain as deposited at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.
6. A product according to any one of claims 1 to 5 comprising per dose, immunoregulatory material from 10^7 to 10^{10} M. vaccae microorganisms.
7. A method of treatment comprising administering an effective non-toxic amount of immunoregulatory material derived from a mycobacterium other than M. tuberculosis and

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of an antigen exogenous to the mycobacterium, to a human or animal in need of T-cell mediated immunity against the exogenous antigen, or otherwise in need of the pattern of T cell-mediated response against the exogenous antigen
5 promoted by the said immunoregulatory material.

8. A method according to claim 7, wherein the immunoregulatory material is as defined in any one of claims 2 to 6.

9. The use in the manufacture of an immunotherapeutic
10 composition for use in treatment of the human or animal body by promoting the T cell-mediated response to an exogenous antigen of immunoregulatory material derived from a mycobacterium other than M. tuberculosis.

10. The use according to claim 9 of immunoregulatory
15 material as defined in any one of claims 2 to 6.

11. A pharmaceutical formulation comprising an association of immunoregulatory material derived from a mycobacterium other than M. tuberculosis and an antigen exogenous to the mycobacterium, and one or more diluents or
20 carriers therefor.

12. A formulation according to claim 11 comprising immunoregulatory material as defined in any one of claims 2 to 6.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 91/01969

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ^o According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl.5 A 61 K 39/39																	
II. FIELDS SEARCHED <div style="text-align: right; margin-right: 100px;">Minimum Documentation Searched⁷</div> <table style="width: 100%; border: none;"> <tr> <td style="width: 25%; border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Classification System</div> </td> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Classification Symbols</div> </td> </tr> <tr> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Int.Cl.5</div> </td> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">A 61 K C 07 K</div> </td> </tr> </table> <div style="text-align: center; margin-top: 10px; font-size: small;"> Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched⁸ </div>			<div style="border: 1px solid black; padding: 2px;">Classification System</div>	<div style="border: 1px solid black; padding: 2px;">Classification Symbols</div>	<div style="border: 1px solid black; padding: 2px;">Int.Cl.5</div>	<div style="border: 1px solid black; padding: 2px;">A 61 K C 07 K</div>											
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III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category^o</th> <th style="width: 70%; padding: 5px;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 20%; padding: 5px;">Relevant to Claim No.¹³</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;"> WO,A,8505034 (UNIVERSITY COLLEGE LONDON) 21 November 1985, see pages 1-3; claims (cited in the application) --- </td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-12</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;"> WO,A,9007935 (AUSPHARM INT. LTD) 26 July 1990, see the claims --- </td> <td style="text-align: center; vertical-align: top; padding: 5px;">1,8,9, 11 2-7,10, 12</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;"> --- International Journal of Leprosy and other Mycobacterial Diseases, vol. 57, no. 1, March 1989 (Bloomfield, NJ, US) R. Ganapati et al.: "A pilot study of three potential vaccines for leprosy in Bombay", pages 33-37, see the summary --- </td> <td style="text-align: center; vertical-align: top; padding: 5px;">2-7,10, 12</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X,P</td> <td style="padding: 5px;"> WO,A,9101751 (UNIVERSITY COLLEGE LONDON) 21 February 1991, see pages 1,2, claims ----- </td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-12</td> </tr> </tbody> </table>			Category ^o	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	X	WO,A,8505034 (UNIVERSITY COLLEGE LONDON) 21 November 1985, see pages 1-3; claims (cited in the application) ---	1-12	X	WO,A,9007935 (AUSPHARM INT. LTD) 26 July 1990, see the claims ---	1,8,9, 11 2-7,10, 12	Y	--- International Journal of Leprosy and other Mycobacterial Diseases, vol. 57, no. 1, March 1989 (Bloomfield, NJ, US) R. Ganapati et al.: "A pilot study of three potential vaccines for leprosy in Bombay", pages 33-37, see the summary ---	2-7,10, 12	X,P	WO,A,9101751 (UNIVERSITY COLLEGE LONDON) 21 February 1991, see pages 1,2, claims -----	1-12
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>^o Special categories of cited documents: ¹⁰</p> <p>^Δ document defining the general state of the art which is not considered to be of particular relevance</p> <p>^T earlier document but published on or after the international filing date</p> <p>^L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>^W document referring to an oral disclosure, use, exhibition or other means</p> <p>^P document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>^T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>^X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>^Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step since the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>^B document member of the same patent family</p> </div> </div>																	
IV. CERTIFICATION <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Date of the Actual Completion of the International Search</div> <div style="text-align: center; margin-top: 10px;">14-02-1992</div> </td> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Date of Mailing of this International Search Report</div> <div style="text-align: center; margin-top: 10px;">16. 03. 92</div> </td> </tr> <tr> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">International Searching Authority</div> <div style="text-align: center; margin-top: 10px;">EUROPEAN PATENT OFFICE</div> </td> <td style="border: none; vertical-align: top;"> <div style="border: 1px solid black; padding: 2px;">Signature of Authorized Officer</div> <div style="text-align: center; margin-top: 10px;"> <div style="display: inline-block; vertical-align: middle;">Maria Pele</div> <div style="display: inline-block; vertical-align: middle; font-family: cursive; font-size: 1.2em; margin-left: 20px;">Hana Pez</div> </div> </td> </tr> </table>			<div style="border: 1px solid black; padding: 2px;">Date of the Actual Completion of the International Search</div> <div style="text-align: center; margin-top: 10px;">14-02-1992</div>	<div style="border: 1px solid black; padding: 2px;">Date of Mailing of this International Search Report</div> <div style="text-align: center; margin-top: 10px;">16. 03. 92</div>	<div style="border: 1px solid black; padding: 2px;">International Searching Authority</div> <div style="text-align: center; margin-top: 10px;">EUROPEAN PATENT OFFICE</div>	<div style="border: 1px solid black; padding: 2px;">Signature of Authorized Officer</div> <div style="text-align: center; margin-top: 10px;"> <div style="display: inline-block; vertical-align: middle;">Maria Pele</div> <div style="display: inline-block; vertical-align: middle; font-family: cursive; font-size: 1.2em; margin-left: 20px;">Hana Pez</div> </div>											
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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim numbers
 Authority, namely:
 Although claims 7 and 8 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound
 because they relate to subject matter not required to be searched by this

2. ☐ Claim numbers
 with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
 because they relate to parts of the international application that do not comply

3. ☐ Claim numbers
 the second and third sentences of PCT Rule 3.4(d).
 because they are dependent claims and are not drafted in accordance with

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This international Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.